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ENDOMETRIOSIS

I405V polymorphism of *CETP* gene and lipid profile in women with endometriosis

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Abstract

Genetic factors have an important role in the pathophysiology of endometriosis. In addition, abnormalities in lipid profile and intrinsic inflammatory status are associated with disease progression. The purpose of this study was to evaluate the effect of the I405V polymorphism of *cholesteryl ester transfer protein* (*CETP*) gene and lipid profile with the risk of endometriosis in women. Ninety-seven women with laparoscopy-diagnosed endometriosis were recruited for this study, and 107 patients with no evidence of endometriosis confirmed by laparoscopy served as controls. Samples were analyzed for polymorphism of the *CETP* gene using polymerase chain reaction–restriction fragment length polymorphism-based methods. After adjustment for body mass index, high-density lipoprotein-C and low-density lipoprotein-C, the risk of endometriosis in patients with normal genotype homozygous was more of the rare allele ($p < 0.001$, odds ratio = 0.21, 95% confidence interval = 0.09–0.45). Our results suggest that I405V polymorphism of *CETP* gene plays an important role as independent factor in the risk of endometriosis in women.

Keywords

CETP, endometriosis, I405V polymorphism, lipid profile

History

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Introduction

Endometriosis is a common benign gynecological disorder that is characterized by the development of the endometrial tissue outside the uterus [1]. Endometrial implants are most commonly found on the visceral peritoneal surfaces. However, the tissue with endometrial abnormalities may also appear on the bladder or bowel [2]. The disease is diagnosed by laparoscopy with or without biopsy for histological diagnosis [2,3]. According to its extent, endometriosis was classified as stages I (minimum), II (mild), III (moderate) and IV (severe) [4]. Despite extensive research, the definitive cause of endometriosis is still unknown [5,6]. Studies showed that elevation of serum lipoprotein in patients with endometriosis is associated with an increased risk of cardiovascular disease [7]. CETP is a key protein in plasma lipoprotein metabolism, particularly as a modulator of high-density lipoprotein (HDL)-C levels. The (*cholesteryl ester transfer protein*) *CETP* gene is located on chromosome 16 and consists of 16 exons [8]. The gene possesses several different single-nucleotide polymorphisms (SNPs) has no sense. One of these polymorphisms is I405V polymorphism (rs5882) that is the substitution of valine to isoleucine at codon 405 in exon 14 [8]. Melo et al. [9] suggested that disruption of lipid profile with elevated low-density lipoprotein (LDL) and non-HDL may increase oxidative stress and inflammation in the peritoneal fluid and increase the risk of endometriosis. This hypothesis supports that women with endometriosis have a dyslipidemia that could provide a suitable pathological substrate for inflammatory process and oxidative stress in endometrial tissue [10]. Several studies evaluated the impact of *CETP* I405V SNP on myocardial infarction risk [11,12], but there are no studies conducted. The purpose of this study was to determine the prevalence of I405V polymorphism of *CETP* gene in women with endometriosis with respect to the control group and concurrent evaluation of this gene polymorphism on lipid profile and the risk of endometriosis.

Materials and methods

Subjects

In this cross-sectional study, women (aged 18–42 years) with chronic pelvic pain or infertility referred to the Kosar Hospital in Qazvin for diagnostic laparoscopy between April 2011 and April 2012 were consecutively included. Among 310 women, 204 were eligible and consented to participation in the study (Figure 1). The study was approved by the ethics committee of Qazvin University of Medical Sciences. To obtain more homogeneous population and verifying that no endometriosis is present, controls were also selected from women undergoing laparoscopy. A total of 97 patients had surgical and histological evidence of endometriosis, while 107 patients without the disease served as controls (women with uterine myoma, dermoid cyst, paraovarian cyst, serous cyst and healthy women). Among the endometriosis patients, 10 patients were diagnosed with stage I, 13 patients with stage II, 35 patients with stage III and 39 patients with stage IV. Endometriosis condition was confirmed by diagnostic laparoscopy or laparotomy in both groups. None of the patients had received hormone therapy during the past 1 year. In addition, women who had received anti-inflammatory drug and

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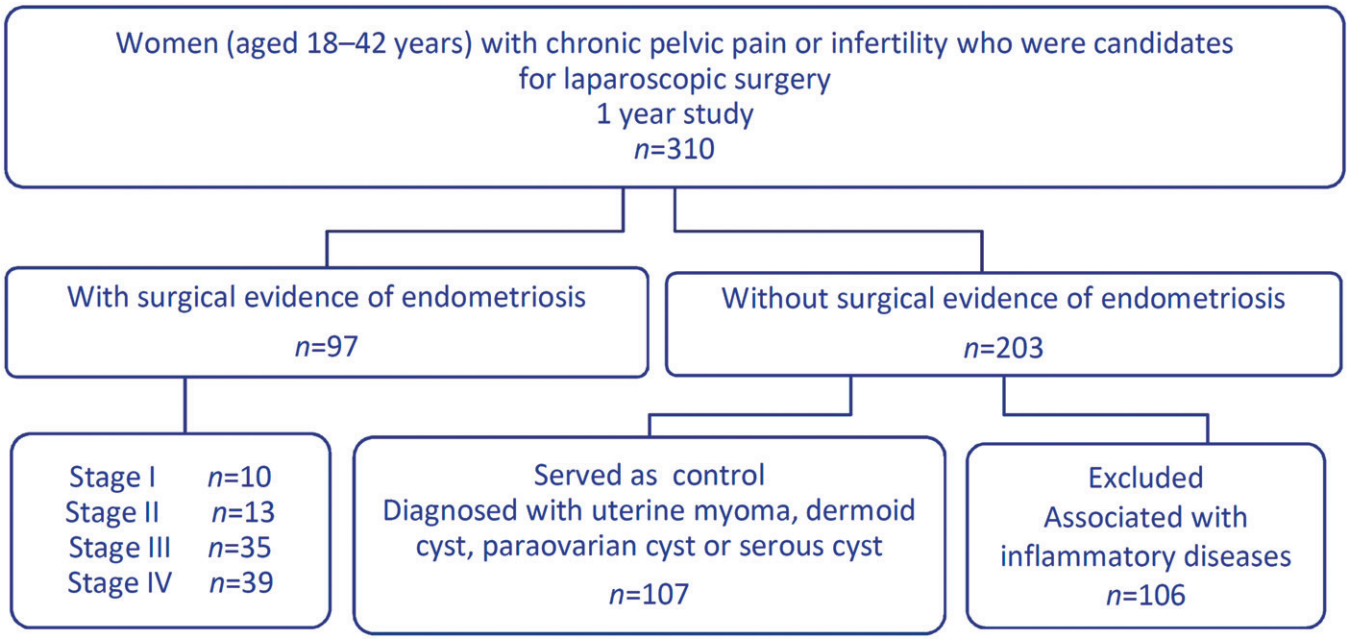

Figure 1. Flowchart of study population.

contraceptives in the past 3 months, or had urological disease, endocrine disorders, familial dyslipidemia and chronic inflammatory were not included in the study.

Lipid profile analysis

Total cholesterol, HDL-C and triglyceride (TG) were determined by standard enzymatic methods. LDL-C was calculated by Friedewald formula: LDL-C = total cholesterol (TC) − (HDL-C + TG/5) [13].

Genotyping

Genomic DNA was extracted from the peripheral blood leukocytes by Qiagen kit (Valencia, CA, USA). A 308-bp sequence of the *CETP* gene was amplified by PCR in a DNA thermal cycle (ABI Veriti, Foster City, CA, USA) by using oligonucleotide primers: F: 5′GCAGAACAGTACTGGCCAAGCAGCG-3′ and R: 5′GCGGTGATCATTGACTGCAGGAAGCTCTGTA-3′.

DNA fragment

The PCR conditions were 96 °C for 5 min, 36 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 6 min [14]. Restriction of the PCR product with the RsaI enzyme results in fragment sizes of 268/40 bp in rare homozygotes, 308/268/40 bp in hetrozygotes and 308 bp in common homozygotes. Samples were electrophoresed in 3% agarose gel, and then the gel was visualized with ethidium bromide.

Statistical analysis

Values were presented as the mean ± standard deviation, and statistical significance was defined as $p < 0.05$. Statistically significant differences in means between genotypes were assessed by *t*-test. Logistic regression analyses were performed to evaluate genotypes with respect to the presence of endometriosis as dependent variable. All analyses were carried out using SPSS 11.0 software (SPSS Inc, Chicago, IL, USA).

Results

Demographic and metabolic parameter of patients and controls are shown in Table 1: mean age (endometriosis: 29.8 ± 5.4 years versus control: 29.5 ± 5.5 years, $p = 0.66$), body mass index (BMI) (endometriosis: 25.1 ± 3.3 kg/m^2 versus control: 26.9 ± 3.9 kg/m^2, $p = 0.001$) and waist circumference (endometriosis: 80.6 ± 9.1 cm versus control: 81.2 ± 9.7 cm, $p = 0.69$). The TC level of the endometriosis group was higher than the control group (216 ± 38 mg/dL versus 175 ± 30 mg/dL, $p < 0.0001$) and also the LDL-C level of the endometriosis group was higher (130 ± 22 mg/dL versus 101 ± 20 mg/dL, $p < 0.0001$). Similarly, HDL-C levels were higher in the endometriosis group (46 ± 10 mg/dL versus 40 ± 9 mg/dL, $p < 0.001$). TG levels were higher in the endometriosis group but were not significantly different between the groups (128 ± 48 mg/dL versus 127 ± 47 mg/dL, $p = 0.86$). The genotype distributions of both groups were in Hardy–Weinberg equilibrium (both $p > 0.05$). Chi-square analysis between genetic groups identified that normal genotypes have more susceptibility than those carrying rare alleles ($p < 0.001$). The distribution of genotypes was different between the endometriosis group and the control group (Table 2). Using logistic regression analysis the risk of endometriosis in different genetic groups was calculated (Table 2). The analysis showed that in normal individuals with homozygous genotype, the risk of endometriosis was more than other groups. Moreover, the analysis, after adjustment factors such as BMI, HDL-C and LDL-C, showed that the risk of endometriosis in patients with normal genotype homozygous was more of the rare allele ($p < 0.001$, odds ratio = 0.21, 95% confidence interval = 0.09–0.45). Analysis of

Table 1. Metabolic parameters of patients with endometriosis versus control.

	Control ($n = 107$)	Endometriosis ($n = 97$)	p
Age, years	29.5 ± 5.5	29.8 ± 5.4	0.66
Body mass index, kg/m^2	26.9 ± 3.9	25.1 ± 3.3	0.001
Waist, cm	81.2 ± 9.7	80.6 ± 9.1	0.69
Cholesterol, mg/dL	175 ± 30	216 ± 38	<0.001
Triglyceride, mg/dL	127 ± 47	128 ± 48	0.86
HDL-C, mg/dL	40 ± 9	46 ± 10	<0.001
LDL-C, mg/dL	101 ± 20	130 ± 22	<0.001

Values are mean ± standard deviation.

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Table 2. Genotype distributions in patients with endometriosis versus control and logistic regression analysis of alleles with respect to the presence of endometriosis as dependent variable.

CETP gene distribution	Control ($n=107$)	Endometriosis ($n=95$)	p			
−/−	33 (30.8%)	69 (72.6%)	<0.001			
+/−	58 (54.2%)	26 (27.4%)				
+/+	16 (15%)	0 (0%)				
Logistic regression	Univariate			Multivariate*		
	OR	95% CI	p	OR	95% CI	p
SNP allele compared to non-carriers						
CETP 405 V	0.17	0.09–0.31	<0.001	0.21	0.09–0.45	<0.001

p values: chi-square tests. Values are 95% confidence interval (CI), odds ratio (OR).
*Adjusted for BMI, HDL-C and LDL-C.

Table 3. Metabolic parameters according to the genotypes.

	CETP I405V			
	II	IV	VV	
n	102	84	16	p
Age, years	29.5 ± 5.1	29.7 ± 5.1	29.6 ± 5.5	0.94
Body mass index, kg/m^2	25.8 ± 3.9	26.0 ± 3.5	27.2 ± 3.3	0.38
Waist, cm	80.7 ± 9.1	81.01 ± 9.5	81.8 ± 10.9	0.91
Cholesterol, mg/dL	207 ± 39	173 ± 37	182 ± 35.8	<0.001
Triglyceride, mg/dL	132 ± 48	120 ± 53	122 ± 45	0.32
HDL-C, mg/dL	43 ± 8.3	38 ± 6.7	43 ± 11	0.22
LDL-C, mg/dL	122 ± 25	103 ± 25	106 ± 22	<0.001

Values are means ± standard deviation, analysis of variance.

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variance showed that in women with endometriosis there is an increase in TC, LDL-C and HDL-C compared to the control ($p<0.001$) (Table 3).

Discussion

Endometriosis is associated with increased inflammation in the peritoneal cavity [15]. In this study, we have shown that women with endometriosis have a dyslipidemia compared to control subjects. These findings were consistent with previous studies [9,10]. Oxidized LDL (oxLDL) can increase endothelial damage and promote the migration of leukocytes and macrophages. Leukocytes and macrophages cause the release of cytokines, platelet accumulation and the release of chemotactic factors [15]. Polak et al. [16] showed an increased oxLDL in the peritoneal fluid of women with endometriosis, in advanced stages.

This finding suggests that endometriosis may increase the risk for atherogenesis that can occur in disease progression; however, this correlation has not yet been confirmed [11]. Our study demonstrated higher HDL-C concentrations in patients with endometriosis.

This study suggested that *CETP* gene polymorphism has important relation with the risk of endometriosis. In this study, the frequency of 405V allele in control group was similar to Asians and more than that in Caucasians and African-American populations [15]. Humans with homozygous *CETP* deficiency have an increased HDL-C levels and decreased LDL-C levels [8,15,17]. Our research has showed that in subjects with I405V polymorphism *CETP* gene, the risk of endometriosis is lower than normal homozygous individuals.

Moreover, our findings suggested that in woman with 405 V allele, TC and LDL-C levels are less than normal homozygous genotype. Cholesterol and LDL-C, as inflammatory risk factors, can be involved in the occurrence and severity of disease, which may indicate beneficial effects of mutation in this gene. The sample size in this study was relatively small due to financial constraints. However, the preliminary results suggest it for further studies. The main limitation of this study was the relatively small sample size and the absence of data on HDL subclass and LDL particle size. Another limitation was that *CETP* activity was not obtained.

In conclusion, this study reported for the first time a significant positive correlation between *CETP* gene polymorphism with the risk of endometriosis. Despite the tendency to decrease endometriosis risk with 405 allele, larger studies are warranted to confirm these observations and to further study the interaction between *CETP* genotype and endometriosis development.

Declaration of interest

The authors report no conflicts of interest. This study was financially supported by the Cellular and Molecular Research Center, Qazvin University of Medical Sciences and the authors acknowledge the Drug Applied Research Center, Tabriz University of Medical Sciences.

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